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REVIEW

Protease-activated receptors and prostaglandins in inflammatory lung disease

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Protease-activated receptors (PARs) are a novel family of G protein-coupled receptors. Signalling through PARs typically involves the cleavage of an extracellular region of the receptor by endogenous or exogenous proteases, which reveals a tethered ligand sequence capable of auto-activating the receptor. A considerable body of evidence has emerged over the past 20 years supporting a prominent role for PARs in a variety of human physiological and pathophysiological processes, and thus substantial attention has been directed towards developing drug-like molecules that activate or block PARs via non-proteolytic pathways. PARs are widely expressed within the respiratory tract, and their activation appears to exert significant modulatory influences on the level of bronchomotor tone, as well as on the inflammatory processes associated with a range of respiratory tract disorders. Nevertheless, there is debate as to whether the principal response to PAR activation is an augmentation or attenuation of airways inflammation. In this context, an important action of PAR activators may be to promote the generation and release of prostanoids, such as prostglandin E_2, which have well-established anti-inflammatory effects in the lung. In this review, we primarily focus on the relationship between PARs, prostaglandins and inflammatory processes in the lung, and highlight their potential role in selected respiratory tract disorders, including pulmonary fibrosis, asthma and chronic obstructive pulmonary disease.
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Abbreviations: AC, adenylate cyclase; ARDS, acute respiratory distress syndrome; BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; COX, cyclooxygenase; DC, dendritic cell; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; mPGES, microsomal prostaglandin E synthase; NK, neurokinin; PAR, protease-activated receptor; PAR-AP, PAR-activating peptide; PGE_2, prostglandin E_2; PGI_2, prostglandin I_2; PGT, prostaglandin transporter; TGF-β, transforming growth factor-β; TNF-α, tumour necrosis factor-α; VCAM, vascular cell adhesion molecule

Introduction

Protease-activated receptors (PARs) are a family of G protein-coupled receptors that possess a unique mechanism of activation (Hollenberg and Compton, 2002; Steinhoff *et al.*, 2005). The emergence of PARs as a novel receptor family was stimulated by the discovery that thrombin specifically cleaves the extracellular N-terminal region of its receptor to create a new receptor amino terminus that functions as an activating tethered ligand (Vu *et al.*, 1991). PAR activation can also occur in the absence of proteolytic activity, by synthetic peptides called PAR-activating peptides (PAR-APs) that mimic the final five to seven amino acids of the tethered ligand sequence. At present, four distinct subtypes of PAR have been characterized and designated PAR_1, PAR_2, PAR_3 and PAR_4, in chronological order of their discovery (Rasmussen *et al.*, 1991; Vu *et al.*, 1991; Nystedt *et al.*, 1995a; Ishihara *et al.*, 1997; Kahn *et al.*, 1998a; Xu *et al.*, 1998). Of particular interest, numerous studies have recently demonstrated the involvement of PARs in a wide variety of physiological and pathophysiological processes (see recent review

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by Ramachandran and Hollenberg, 2008). The actions of PARs and their activating proteinases in the airways have also been studied extensively to determine their role in various lung diseases (Sokolova and Reiser, 2007). Among their actions, PARs induce cyclooxygenase (COX) activation and expression in a variety of cell types, resulting in the synthesis and release of various prostanoids (Cocks *et al.*, 1999). PAR-mediated prostaglandin production represents an attractive target for the development of treatments for inflammatory lung diseases, and is the principal focus of this review.

Expression and signalling of PARs in the lung

The human respiratory tract appears to express all four PAR subtypes. Within the lung, PARs are expressed on many cell types including alveoli, fibroblasts, airway smooth muscle, nerves, epithelial cells, endothelial cells, mesothelial cells, goblet cells, as well as on various leukocytes (Sokolova and Reiser, 2007; Ramachandran and Hollenberg, 2008). Of particular interest, exposure of the lung to inflammatory stimuli may enhance the expression of PARs, as well as of PAR-activating proteases. Proteases that may be present in the respiratory tract and activate PARs include the endogenous enzymes mast cell tryptase (activates PAR_2), trypsin (PAR_1, PAR_2 and PAR_4), chymase (PAR_1) and cathepsin G (PAR_4), as well as exogenous enzymes such as Der p1 (PAR_2) that are inhaled. However, these and other enzymes within the respiratory tract may also inactivate or disarm various PARs by cleaving them at other sites that remove the tethered ligand sequence (Loew *et al.*, 2000).

PAR_1 is the primary cell-surface receptor responsible for thrombin-mediated platelet aggregation in humans (Rasmussen *et al.*, 1991; Vu *et al.*, 1991; Ahn *et al.*, 2000). PAR_1 is also activated by many other proteases, including activated protein C (APC), and by selective PAR_1-APs such as TFLLR-NH_2 (Vu *et al.*, 1991; Ramachandran and Hollenberg, 2008). Non-peptide agonists for PAR_1 are currently unavailable. Nevertheless, several potent and selective non-peptidic PAR_1 antagonists have been developed (Ramachandran and Hollenberg, 2008). One such antagonist, SCH530348, is currently undergoing phase III clinical trials as a preventative medication for atherothrombosis (Clasby *et al.*, 2007; Butler, 2008; Severino *et al.*, 2008).

PAR_1 is expressed on many cell types within the lungs, including airway smooth muscle, epithelial cells, platelets, macrophages, mast cells, $CD3^+$ T lymphocytes and fibroblasts (Knight *et al.*, 2001; Hollenberg and Compton, 2002; Lan *et al.*, 2002; Steinhoff *et al.*, 2005; Li and He, 2006; Sokolova and Reiser, 2007; Ramachandran and Hollenberg, 2008). Although PAR_1 expression does not appear to be altered in asthmatic airways, increased expression is seen following exposure of cells to influenza A, cockroach allergen and various PAR-APs (Knight *et al.*, 2001; Lan *et al.*, 2004; Ostrowska *et al.*, 2007; Zhang *et al.*, 2008). PAR_1 expression is decreased in fibroblasts following PGI_2 or prostglandin E_2 (PGE_2) exposure (Sokolova *et al.*, 2005), possibly involving a cAMP-dependent mechanism as demonstrated in vascular smooth muscle cells (Sokolova *et al.*, 2005; Pape *et al.*, 2008). $G_{q/11}$ appears to be the primary signalling G protein for PAR_1, although it has also been reported to signal via G_i and $G_{12/13}$ pathways depending on cell type (see review by Ramachandran and Hollenberg, 2008).

The cloning and characterization of PAR_1 was followed by the discovery of PAR_2, which was shown to be activated by trypsin but resistant to thrombin (Nystedt *et al.*, 1995a,b). In addition to endogenous proteases such as trypsin and tryptase, PAR_2 can be activated by exogenous proteases, such as Der p1 from the house dust mite Dermatophagoides Pteronyssinus and by PAR_2-APs such as SLIGKV-NH_2 (Molino *et al.*, 1997; Asokananthan *et al.*, 2002; Page *et al.*, 2003). Selective small-molecule agonists, as well as an antagonist, have recently been developed; however, they have low potency and are not yet widely available (Kelso *et al.*, 2006; Gardell *et al.*, 2008; Seitzberg *et al.*, 2008).

PAR_2 is expressed on a range of cell types including mesothelial cells of the pleura, bronchial glands, epithelial cells, endothelial cells, smooth muscle cells, nerves and immune cells such as $CD3^+$ T lymphocytes, eosinophils, mast cells and neutrophils (Knight *et al.*, 2001; Miotto *et al.*, 2002; Henry, 2006; Li and He, 2006). PAR_2 expression is elevated following exposure to a variety of inflammatory stimuli, including respiratory tract viruses, smoke, bacterial products and allergens (Knight *et al.*, 2001; Ostrowska *et al.*, 2007). PAR_2 signals primarily through activation of $G_{q/11}$, but has recently been shown to signal through G protein-independent mechanisms via β-arrestins (Zoudilova *et al.*, 2007).

The discovery of a second platelet thrombin receptor (Connolly *et al.*, 1996) was soon followed by the cloning and characterization of PAR_3 (Ishihara *et al.*, 1997). PAR_3 mRNA is expressed in airway smooth muscle cells, epithelial cells, $CD3^+$ T lymphocytes and fibroblasts (Hauck *et al.*, 1999; Shimizu *et al.*, 2000; Li and He, 2006; Ramachandran *et al.*, 2006), and its expression on epithelial cells is increased following exposure to influenza A. Somewhat surprisingly, the corresponding tethered ligand sequence does not activate PAR_3. At first it appeared that proteolytic cleavage of PAR_3 and exposure of its tethered ligand did not induce signalling *per se*; rather PAR_3 appeared to act as a co-factor for the activation of other PARs (Kahn *et al.*, 1998b). For example, PAR_3 dimerized with PAR_1 and PAR_4 to amplify their signalling (Nakanishi-Matsui *et al.*, 2000; Weiss *et al.*, 2002; McLaughlin *et al.*, 2007). A recent study, however, has shown that thrombin can signal through PAR_3 independently of other PARs, to induce interleukin (IL)-8 release from HEK-293 cells, via ERK1/2 phosphorylation (Ostrowska and Reiser, 2008).

The observation that thrombin could stimulate the aggregation of mouse platelets in the absence of PAR_1 and PAR_3 provided evidence for the existence of a fourth PAR subtype (Kahn *et al.*, 1998b; Xu *et al.*, 1998). Although thrombin activates PAR_4, it is much less potent at PAR_4 than at PAR_1 (Kahn *et al.*, 1998b; Xu *et al.*, 1998). A PAR-AP, HYPGKF-NH_2 also activates PAR_4. No non-peptidic agonists for PAR_4 are available; however, a non-peptidic antagonist has been reported (Wu *et al.*, 2002). A low potency peptide antagonist has been developed, although it exhibits low selectivity and is known to produce non-PAR effects (Hollenberg and Saifeddine, 2001; Hollenberg *et al.*, 2004). P4pal-10 is a

high-potency pepducin antagonist for PAR_4, although it also partially inhibits activation of PAR_1 by SFLLRN-NH_2 (Covic *et al.*, 2002a,b; Kuliopulos and Covic, 2003; Hansen *et al.*, 2008).

PAR_4 is widely expressed in the lung, present on endothelial and epithelial cells, airway smooth muscle cells as well as alveolar macrophages (Lan *et al.*, 2000; Shimizu *et al.*, 2000; Asokananthan *et al.*, 2002; Kataoka *et al.*, 2003). PAR_4 expression on bronchial fibroblasts was recently reported to be elevated following exposure to inflammatory stimuli, such as tumour necrosis factor-α (TNF-α) (Ramachandran *et al.*, 2007). PAR_4 signals through a $G_{q/11}$-mediated pathway (Xu *et al.*, 1998).

PAR-mediated effects in the lung

PAR-mediated inhibition of airway smooth muscle tone

PAR_2 activators can modulate bronchomotor tone, with the predominant effect being bronchodilatation. For example, intravenous administration of PAR_2-APs attenuated methacholine-, serotonin- and histamine-induced increases in airway resistance in mice, rats and guinea pigs respectively (Cicala *et al.*, 1999; Cocks *et al.*, 1999; Lan *et al.*, 2004). Consistent with this, PAR_2-APs induce concentration-dependent relaxation response in isolated airway preparations from these animals (Cocks *et al.*, 1999; Chow *et al.*, 2000; Lan *et al.*, 2000; Ricciardolo *et al.*, 2000; Kawabata *et al.*, 2004b; Franchi-Micheli *et al.*, 2005). In general, inhibitors of COX such as indomethacin block PAR_2-induced bronchodilatory effects, indicating a prominent mediator role for relaxant prostanoids in this response (Cicala *et al.*, 1999; Cocks *et al.*, 1999; Lan *et al.*, 2004). Direct evidence that PGE_2 was an important mediator in PAR-induced relaxation responses came from studies showing that exposure of murine airways to PAR_2-APs caused concentration-dependent increases in PGE_2 release, which correlated strongly and positively with the magnitude of the relaxation response (Lan *et al.*, 2001). PAR_2-mediated release of PGE_2 is likely to cause bronchodilator responses via activation of airway smooth muscle EP_2 receptors, which signal through $G_{\alpha s}$, adenylate cyclase and cAMP (Fortner *et al.*, 2001; Lan *et al.*, 2001).

PAR-mediated production of the anti-inflammatory prostanoid PGE_2

In most organ systems, PGE_2 promotes inflammatory processes, whereas it produces predominantly anti-inflammatory effects in the lung (Vancheri *et al.*, 2004). This section will cover the specific prostaglandins released by PAR subtypes, the mechanisms involved in PAR-mediated generation of prostaglandins and the modulatory effects of these prostaglandins on inflammatory processes in the airways. As indicated above, PAR activators induce the rapid and sustained formation and release of prostanoids from a wide variety of cell and tissue types. For example, PAR_1-APs cause PGE_2 release from human bronchial epithelial cells (Asokananthan *et al.*, 2002) and human lung fibroblasts (Sokolova *et al.*, 2005; Sokolova *et al.*, 2008). Human bronchial airway epithelial cell cultures release PGE_2 following exposure to PAR_2-APs; however, cultured airway smooth muscle cells, while capable of PGE_2 production, do not do so in response to PAR_2 activation (Pang and Knox, 1997; Asokananthan *et al.*, 2002; Chambers *et al.*, 2003; Dulon *et al.*, 2005; Sharma *et al.*, 2006). Tryspin induces PGE_2 release from airway smooth muscle cells independently of PAR_2, suggesting that PAR_4 may induce PGE_2 release from airway smooth muscle (Chambers *et al.*, 2003). PAR_4-APs can also induce PGE_2 release from bronchial epithelial cell cultures (Asokananthan *et al.*, 2002). PAR_1, PAR_2 and PAR_4-APs increased PGE_2 levels in murine isolated tracheal preparations, with PAR_2-APs inducing the largest response (Lan *et al.*, 2001). As PAR_2 expression on airway epithelial cells increases in response to a variety of inflammatory stimuli, PAR_2-mediated generation of PGE_2 may be amplified in inflammatory lung disorders (Knight *et al.*, 2001).

PAR-mediated PGE_2 production occurs via a complex signalling pathway, which has been studied in greatest detail in cultures of A549 cells (a transformed human airway epithelial cell line) (Kawao *et al.*, 2005), and in mouse isolated tracheal preparations (Kawabata *et al.*, 2004b). In these systems, PAR_2-APs induce a rapid, transient increase in PGE_2 levels, which is thought to sequentially involve the activation of PAR_2, an increase in $[Ca^{2+}]_i$, activation of cytosolic phospholipase A_2, release of arachidonic acid and downstream processing of arachidonic acid by COX-1 and prostaglandin E synthase (PGES). This is followed by a second, sustained phase of PGE_2 generation involving COX-1-dependent up-regulation of COX-2 and microsomal prostaglandin E synthase-1 (mPGES-1) (Kawabata *et al.*, 2004b; Kawabata and Kawao, 2005; Kawao *et al.*, 2005; Nagataki *et al.*, 2007; Sekiguchi *et al.*, 2007; Hsieh *et al.*, 2008; Rastogi *et al.*, 2008; Wang *et al.*, 2008). PAR_2-mediated production of PGE_2 appears to be dependent on mPGES-1 but not mPGES-2 or cPGES (Nagataki *et al.*, 2007). Figure 1 shows a simplified representation of PAR_2-mediated generation of PGE_2.

PGE_2 induces an extensive array of effects within the respiratory tract (Table 1), affecting the activity of most structural and inflammatory cells. These effects are mediated by E prostanoid receptors, a family of four G protein-coupled receptors (EP_1–EP_4) linked to $G_{q/11}$ (EP_1 receptors), G_s (EP_2 and EP_4 receptors) and $G_{i/o}$ (EP_3 receptors) (Alexander *et al.*, 2008). As highlighted by Vancheri and coworkers (Vancheri *et al.*, 2004) and the data presented in Table 1, PGE_2 appears to have a role in limiting the immune-inflammatory response and tissue repair processes. These findings indicate that PAR-mediated production of PGE_2 may be beneficial in instances when dysregulated inflammatory and tissue repair processes contribute to disease.

PAR-mediated production of pro-inflammatory cytokines

PAR activation may also promote inflammatory processes in the respiratory tract, as indicated by reports that PAR-APs induce the release of pro-inflammatory cytokines and mediators in cell cultures relevant to airways disease. For example, PAR_1, PAR_2 and PAR_4-APs induced IL-6 and IL-8 release from airway epithelial cells (Asokananthan *et al.*, 2002).

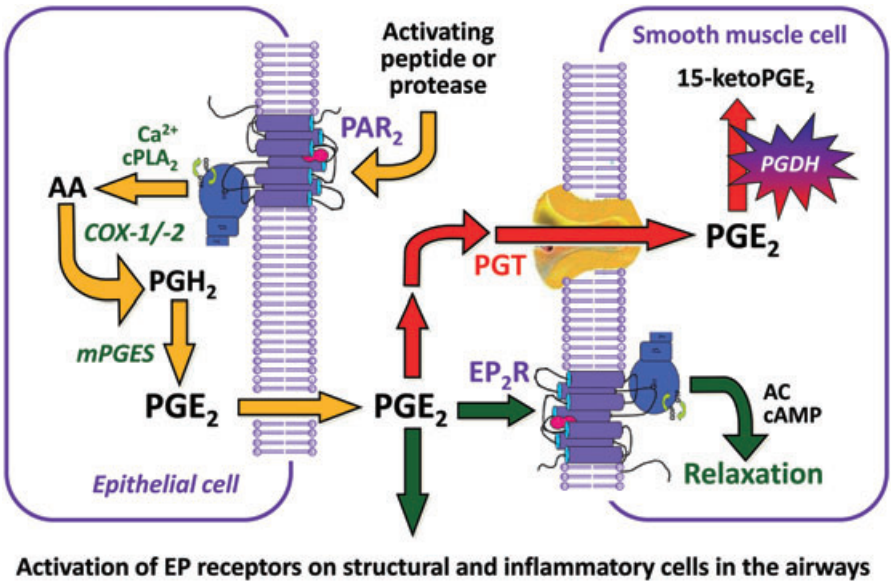

Figure 1 PAR_2-mediated generation of PGE_2 in the airways.

Additionally, PAR_1- and PAR_2-APs, but not PAR_3- or PAR_4-APs, induced IL-6, but not IL-13, release from cultured human T cells (Li and He, 2006). PAR_1 activators have also been reported to induce CCL2, IL-1β and TNF-α release from macrophages (Naldini *et al.*, 1998; 2002; Riewald *et al.*, 2002; Colognato *et al.*, 2003), and PAR_2-APs induced MMP-9 release from primary cultures of human airway epithelial cells (Vliagoftis *et al.*, 2000). The PAR_2-AP, SLIGKV-NH_2, induced GM-CSF mRNA expression but did not increase protein expression in human fibroblasts. SLIGKV-NH_2 also increased cell surface expression of VCAM-1 on primary human bronchial fibroblasts (Ramachandran *et al.*, 2006). Furthermore, PAR_2 activation promoted eosinophil degranulation and superoxide production (Miike *et al.*, 2001), and may promote IL-8 release (Temkin *et al.*, 2002).

PAR-mediated modulation of allergic, eosinophilic inflammation

Consistent with PAR-mediated production of pro-inflammatory cytokines *in vitro*, several *in vivo* studies have demonstrated a pro-inflammatory role for PARs in the airways. For example, mice lacking PAR_2 exhibit decreased inflammation in an allergen challenge model, whereas over-expression of PAR_2 was associated with increased inflammation (Schmidlin *et al.*, 2002; Takizawa *et al.*, 2005). In subsequent studies, co-administration of PAR_2-APs caused increased cell influx, IL-5, IL-13 and TNF-α in bronchoalveolar lavage (BAL) fluid, while lowering IL-10, in allergen-sensitized and challenged mice (Ebeling *et al.*, 2005; 2007). In these latter studies, a single dose of PAR_2-AP did not induce an inflammatory response; however, multiple doses did. Other studies have suggested that PAR_2 may induce airway inflammation through a neuropeptide-dependent mechanism; however, this requires further investigation given that PAR_2-APs are known to activate NK_1 receptors (Su *et al.*, 2005; Abey *et al.*, 2006).

On the contrary, there is also a significant body of evidence indicating that PAR activators suppress inflammatory processes within the lung. Of particular relevance to the current review, the anti-inflammatory effects produced by PAR activators appear to be mediated by prostaglandins such as PGE_2 (Asokananthan *et al.*, 2002; Henry, 2006). For example, intratracheally administered PAR_2-APs reduced bronchoconstriction, airway hyperresponsiveness and eosinophil influx in a murine model of allergic inflammation (De Campo and Henry, 2005). This PAR-mediated effect was suppressed by inhibitors of COX and mimicked by PGE_2 (De Campo and Henry, 2005). Administration of PAR_2-AP to antigen-sensitized and challenged rabbits was also associated with reduced bronchoconstriction, airway hyperresponsiveness and eosinophilia (D'Agostino *et al.*, 2007), although the role of COX and PGE_2 is uncertain. Nevertheless, this latter study showed a marked increase in IL-10 mRNA from $CD4^+$ T cells recovered from PAR_2-AP-treated animals, as well as decreased interferon (IFN)-γ and IL-2 production to control levels (D'Agostino *et al.*, 2007), consistent with an anti-inflammatory response.

Thus, a puzzling, yet not uncommon trait of research investigating the role of PARs in inflammatory processes in the respiratory system is the existence of seemingly contradictory observations, with some reports demonstrating that PAR-APs induce inflammatory responses, and others demonstrating overt anti-inflammatory effects. Although there is unlikely to be single reason that adequately explains these inconsistencies, a contributing factor is likely to be the lack of subtype-selective small-molecule agonists and antagonists for PARs, often necessitating the use of indirect methods to determine the role of PARs in these processes.

As PAR-APs are typically between five and seven amino acids long, the development of small-molecule lead compounds that selectively bind to PARs is problematic. Thus, there is a current paucity of agents capable of selectively activating and inhibiting these receptors. Until recently, this has necessitated the use of one or more of the following

Table 1 Summary of effects produced by prostglandin E_2 (PGE_2) in isolated cells of the respiratory tract, in animal models of airway disease, and in humans with airway disease

	Response to PGE_2	*EP receptor (species if not human)*	*Reference*
In vitro effects of PGE_2 on			
Airway smooth muscle	Relaxation	EP_2	Norel *et al.* (1999)
	↓Proliferation	EP_2	Burgess *et al.* (2004); Kassel *et al.* (2008)
	↓Migration	?	Goncharova *et al.* (2003)
	↓RANTES, ICAM, GM-CSF, Il-8, eotaxin, MCP-1	?	Ammit *et al.* (2000); Lazzeri *et al.* (2001); Wuyts *et al.* (2003); Kaur *et al.* (2008)
	↑VEGF, G-CSF, Il-6	EP_2/EP_4	Ammit *et al.* (2000); Bradbury *et al.* (2005); Clarke *et al.* (2005)
Epithelial	↑Cilia beat frequency	?	Bonin *et al.* (1992); Schuil *et al.* (1995); Haxel *et al.* (2001)
	↑Cl-channel conductance	EP_4 (frog, cow)	Clayton *et al.* (2005); Palmer *et al.* (2006); Joy and Cowley (2008); Seto *et al.* (2008)
	↑Na^+ transport	EP_1/EP_2 (frog)	Berk *et al.* (2004)
	↑Mucin secretion	EP_4	Kook Kim *et al.* (2006)
	↑MUC5A/8 expression	EP_4	Cho *et al.* (2005); Kook Kim *et al.* (2006); Song *et al.* (2009)
	↑Rate of wound closure	$EP_1/EP_4/EP_2$?	Savla *et al.* (2001)
	↓Il-8 & ET-1 secretion	EP_3/EP_4	Pelletier *et al.* (2001); Hattori *et al.* (2008)
	↑Il-6 release	EP_2/EP_4	Tavakoli *et al.* (2001)
Submucosal gland	↑Ionic currents & secretory function	? (pig)	Liu *et al.* (2005)
	↑Sensitivity to acetylcholine	EP_2 (pig)	Liu and Farley (2007)
Cholinergic nerve	↓Acetylcholine release	EP_3 (guinea pig, dog)	Deckers *et al.* (1989); Zhao *et al.* (1994); Spicuzza *et al.* (1998); Clarke *et al.* (2004)
Sensory nerve (pulmonary C-fibre afferents)	↑Sensitivity to chemical stimulants	EP_2 (rat)	Ho *et al.* (2000); Kwong and Lee (2002; 2005)
Alveolar type II	↑Surfactant secretion	EP_1 (rat)	Marino and Rooney (1980); Morsy *et al.* (2001)
	↑Na^+ uptake	EP_3	Mukhopadhyay *et al.* (1998)
Pulmonary endothelial	↑Barrier function		Birukova *et al.* (2007)
Alveolar macrophage	↓Phagocytosis	EP_2 (mouse/rat)	Canning *et al.* (1991); Aronoff *et al.* (2004); Canetti *et al.* (2007); Brock *et al.* (2008); Lee *et al.* (2009); Medeiros *et al.* (2009)
	↓Bacterial killing	EP_2/EP_4	Serezani *et al.* (2007)
	↓Mitochondrial inner membrane perturbation and necrosis	EP_2	Chen *et al.* (2008)
	↓TNF-α	EP_2/EP_4	Ratcliffe *et al.* (2007)
	↑Il-10 & NO release	? (rat)	Menard *et al.* (2007)
Fibroblast	↓proliferation	EP_2	Bitterman *et al.* (1986); Liu *et al.* (2004); Huang *et al.* (2007); Huang *et al.* (2008)
	↓Collagen production	EP_2	Saltzman *et al.* (1982); Fine *et al.* (1989); Liu *et al.* (2004); Huang *et al.* (2007); Huang *et al.* (2008)
	↓Fibroblast to myofibroblast transition	?	Kolodsick *et al.* (2003)
	↓Myofibroblast differentiation		Dunkern *et al.* (2007)
	↓Migration	EP_2	Kohyama *et al.* (2001); White *et al.* (2005)
	↓Smoke-induced apoptosis	EP_2	Sugiura *et al.* (2007)
Mast cell	↓Migration	EP_3?	Duffy *et al.* (2008)
	↓Histamine release	EP_2	Drury *et al.* (1998); Kay *et al.* (2006); Duffy *et al.* (2008)
Dendritic cell	↑Podosome dissolution		van Helden *et al.* (2008)
	↑Migration	EP_2/EP_4	Legler *et al.* (2006)
	↑Maturation	EP_2/EP_4	Kubo *et al.* (2004)
	↓CCL3 and CCL4	EP_2/EP_4 (mouse)	Jing *et al.* (2003)
	↑Resistance to apoptosis	EP_2/EP_4	Baratelli *et al.* (2005a)
	↓TNF-α release & antigen presentation	? (mouse)	Kambayashi *et al.* (2001)
T cells	↓Proliferation	?	Jarvinen *et al.* (2008)
	↑Il-10 expression	?	Benbernou *et al.* (1997)
T_{reg}	↑Inhibitory function & differentiation	?	Baratelli *et al.* (2005b)
	↑Expansion	?	Garg *et al.* (2008)
Eosinophils	↓Migration	EP_2/EP_4	Sturm *et al.* (2008)
	↓Release from bone marrow	? (guinea pig)	Sturm *et al.* (2008)
	↓Degranulation	EP_2/EP_4	Sturm *et al.* (2008)
	↓PAF-induced aggregation	EP_2 (guinea pig)	Teixeira *et al.* (1997)

Table 1 *Cont.*

	Response to PGE_2	*EP receptor (species if not human)*	*Reference*
Neutrophils	↓Chemotaxis	EP_2?	Armstrong (1995)
B cells	↓Proliferation	EP_4 (mouse)	Murn *et al.* (2008)
	Promotes differentiation and Il-4 and LPS-driven class switching to IgE	EP_2/EP_4 (mouse)	Fedyk and Phipps (1996)
In vivo effects of PGE_2 in			
Normal animals or subjects	Bronchodilatation	EP_2 (mouse)	Mathe and Hedqvist (1975); Smith *et al.* (1975); Sheller *et al.* (2000); Tilley *et al.* (2003)
Asthmatics (allergic)	Bronchodilatation		Smith *et al.* (1975)
	↓Early and late response to allergen		Pavord *et al.* (1993); Gauvreau *et al.* (1999)
	↓Airway hyperresponsiveness and sputum eosinophils		Gauvreau *et al.* (1999)
	↓BAL PGD_2, ↓BAL eosinophils		Hartert *et al.* (2000)
Asthmatic (exercise)	↓Exercise-induced bronchoconstriction		Melillo *et al.* (1994)
Allergic inflammation	↓Allergen-induced bronchoconstriction	EP_2/EP_4 (guinea pig)	Martin *et al.* (2002); Tanaka *et al.* (2005)
	↓BAL eosinophils	? (mouse, rat)	Martin *et al.* (2002); De Campo and Henry (2005); Sturm *et al.* (2008)
	↓BAL LTs, ↓T cell cytokine expression	? (rat)	Martin *et al.* (2002)
Bleomycin-induced fibrosis	PGE_2 synthetic analogue 16,16-dimethyl-PGE_2 ↓infiltration by leukocytes, ↓myeloperoxidase activity, ↓Il-1, TNF-α and nitrotyrosine, ↓lung edema & injury, ↓collagen deposition, ↓weight loss and mortality	? (mouse)	Failla *et al.* (2009)

Not all references to PGE_2-induced responses are cited, with preference given to studies that have used human cells, tissues or subjects. Animal studies are cited where human studies have not been reported, or to indicate which E-prostanoid (EP) receptor subtype mediated the response.

approaches to explore PARs: (i) use of PAR-activating proteases; (ii) use of relatively selective but low-potency PAR-APs; and (iii) manipulation of PAR expression.

Although numerous proteases are well-established activators of PARs, their use in characterizing subtypes of PARs is often limited. One particular limitation is that proteases frequently possess a multitude of effects – being capable of activating more than one PAR subtype as well as inducing PAR-independent effects. For example, thrombin can activate PAR_1, PAR_3 and PAR_4, as well as induce smooth muscle proliferation in a PAR-independent fashion (Tran and Stewart, 2002). The currently used PAR-APs are typically more selective than proteases, but most lack absolute subtype specificity and may activate non-PAR receptors (Abey *et al.*, 2006). Furthermore, aminopeptidases can degrade most PAR-APs, which limits their effectiveness. An exception is the aminopeptidase-resistant PAR_2-AP 2-furoyl-LIGRLO-NH_2 (Kawabata *et al.*, 2004a). Gene knockout and overexpression approaches have been used to investigate the role of PARs, although the ubiquitous expression of PARs, and their central role in platelet aggregation, coagulation and inflammation makes clear interpretation of findings difficult. The development of potent, subtype-selective, small-molecule ligands for PARs will provide valuable information on the roles of these receptors, and may help clarify current inconsistencies in the airway PAR literature.

Potential role of PARs in asthma

Asthma is a chronic inflammatory airway disease, characterized by shortness of breath and repeated wheezing episodes (Masoli *et al.*, 2004; Hamid and Tulic, 2009). Allergic asthma involves an inappropriately large immune response to one or more inhaled allergens (Busse and Lemanske, 2001). In asthmatic individuals, otherwise innocuous stimuli trigger a Th_2 cell-driven immune response frequently involving antigen-specific IgE production, release of mast cell-derived mediators and recruitment of eosinophils to the airways. Allergen-induced inflammation is typically associated with acute bronchoconstriction, airway hyperresponsiveness and eventually, airway remodelling. The current mainstay of asthma treatment remains glucocorticoids as a preventative medication, with short- or long-acting β_2-adrenoceptor agonists as a reliever from acute attacks (Adcock *et al.*, 2008). Recently, newer medications such as anti-leukotrienes and IgE inhibitors have been used in the clinical management of asthma (Holgate and Polosa, 2008). While many of the current medications are useful in managing the clinical symptoms of asthma, they are not curative and the search for better asthma treatments remains an important focus.

Increased immunization, antibiotic use, altered diet and decreased pathogen exposure contribute to an immune system that is more susceptible to developing allergies

(Strachan, 1989; Anderson *et al.*, 2001; Kaiser, 2004; Devereux, 2006). Of the immune cells involved in allergic asthma, the $CD4^+$ T cells appear to play a central role in the development and maintenance of allergic sensitization (Fischer *et al.*, 2007; Anderson, 2008; Burchell *et al.*, 2008; Pucci and Incorvaia, 2008). $CD4^+$ T cells can be broadly categorized into four subsets, namely, T helper type 1 (Th_1), Th_2, Th_{17} and regulatory T cells (T_{reg}). Subtype selection of undifferentiated T cells (Th_0) is controlled by the cytokine signals present upon stimulation of the Th_0 cell (Murphy and Reiner, 2002; Curiel, 2007; McGeachy and Cua, 2008; Korn *et al.*, 2009). These cells serve varied and distinct functions in immune responses (Broide, 2008). Th_1 cells are primarily responsible for coordinating the immune response to intracellular infections such as viruses (Murphy and Reiner, 2002). Th_2 cells are primarily involved in the destruction of extracellular pathogens such as helminth parasites (Fallon and Mangan, 2007). Th_{17} cells have not been studied as extensively due to their recent discovery; however, an increasing body of evidence suggests their involvement in asthma and allergy (Infante-Duarte *et al.*, 2000; Korn *et al.*, 2008; Lochner *et al.*, 2008; McKinley *et al.*, 2008; Oboki *et al.*, 2008). T_{reg} cells suppress inflammation and are involved in maintaining peripheral tolerance (Cohn, 2008; Vignali *et al.*, 2008). A polarization towards Th_2-type responses appears central to the pathogenesis of asthma (Salvi *et al.*, 2001; O'Byrne *et al.*, 2004; Strickland *et al.*, 2006; Galli *et al.*, 2008; Oboki *et al.*, 2008; Pucci and Incorvaia, 2008; Broide, 2009; Hamid and Tulic, 2009). While recent evidence suggests that Th_2 cells are not the only T helper lineage involved in allergic asthma, they play a significant role and therefore, inhibiting their actions may prove useful in asthma (Holt *et al.*, 2005; Fischer *et al.*, 2007; Holt and Sly, 2007; Caramori *et al.*, 2008; Schmidt-Weber, 2008). Very few controlled clinical trials have determined the effect of currently used medications on Th_2 cell responses (Caramori *et al.*, 2008).

PAR activation may alter $CD4^+$ cell polarization in the airways and inhibit allergic inflammation through a variety of mechanisms, including via production of PGE_2. While PGE_2 favours Th_1 differentiation *in vitro*, the converse appears to be true *in vivo* (Betz and Fox, 1991; Snijdewint *et al.*, 1993; Martin *et al.*, 2002; Nagamachi *et al.*, 2007). Administering PGE_2 *in vivo* appears to inhibit Th_2 activation and cytokine expression (Martin *et al.*, 2002), and inhibits T cell proliferation through EP_2 receptors (Nataraj *et al.*, 2001). PGE_2 also inhibits transendothelial migration of lymphocytes into the airways, likely via increased intracellular cAMP (Pober *et al.*, 1993; Oppenheimer-Marks *et al.*, 1994; Panettieri *et al.*, 1995). Consistent with this, mice deficient in COX-1 or COX-2 show increased inflammation upon allergen challenge, and inhibitors of COX-1 or COX-2 produce similar effects (Gavett *et al.*, 1999; Stokes Peebles *et al.*, 2002; Carey *et al.*, 2003). Epidemiological studies have also shown that frequent use of COX inhibitors may increase the risk of developing asthma and allergy (Allmers, 2005). Furthermore, a recent study has shown that exposing mice to indomethacin during allergic sensitization increases primary and memory Th_2 responses *in vivo* (Zhou *et al.*, 2008). Thus, the activation and up-regulation of COX may well increase prostaglandin levels in the lung, thereby reducing Th_2 polarization and inflammation.

Elevating PGI_2 levels represents another distinct pathway by which PARs could alter T helper cell polarization in the airways. Activation of endothelial PAR_1- and PAR_2-APs promotes PGI_2 release (Syeda *et al.*, 2006), which may alter Th_2 immune function by stimulating Th_2 cells to release IL-10, an anti-inflammatory cytokine that serves to suppress immune responses (Jaffar *et al.*, 2002). Additionally, PGI_2 markedly inhibits CCL17-induced chemotaxis of Th_2 cells, perhaps by inhibiting CCR4 and/or CCR8 signalling, resulting in fewer Th_2 cells being recruited to the airways following allergen challenge (Jaffar *et al.*, 2007). Furthermore, pre-treating Th_2 cells with PGI_2 before adoptive transfer markedly decreased inflammation in mice following allergen challenge (Jaffar *et al.*, 2007). Interestingly, Th_2 cells exhibit increased expression of prostanoid IP receptors compared with Th_1 cells. This allows for selective inhibition of Th_2 cells by PGI_2, which may reduce the ratio of Th_2 cells present in the airways (Jaffar *et al.*, 2002). Thus, PAR-mediated production of PGI_2 may selectively inhibit Th_2 immune responses.

IP receptor-deficient mice exhibit increased eosinophil, lymphocyte and neutrophil influx in an allergen challenge model. These IP-null mice also had increased total and antigen-specific serum levels of IgE as well as total IgG. Allergen challenge of isolated spleenocytes from these IP-null mice resulted in increased IL-4 compared with wild-type mice (Takahashi *et al.*, 2002). These findings are supported by studies using an IP receptor agonist, iloprost, in an allergen challenge model. Iloprost decreased allergen-induced BAL fluid levels of eosinophils, lymphocytes, IL-4, IL-5 and IL-13; and these effects were abolished by an IP receptor antagonist. Additionally, iloprost inhibited allergen-induced increases in airway resistance, as well as decreases in compliance (Idzko *et al.*, 2007). Iloprost also altered dendritic cell (DC) function. DCs incubated with iloprost and then adoptively transferred to mice showed markedly decreased Th_2-like responses upon subsequent allergen challenge compared with vehicle-treated DCs. Iloprost treatment of DCs prior to adoptive transfer inhibited allergen-induced BAL numbers of macrophages, lymphocytes and eosinophils; as well as inhibiting IL-4, IL-5 and IL-13; and increasing IL-10 and IFN-γ production (Idzko *et al.*, 2007). Thus, PGI_2 decreases Th_2 cell mediator release, DC induction of Th_2 cell differentiation and allergen-induced cell influx. Additionally, PGI_2 increases levels of the anti-inflammatory cytokine IL-10. Thus, agents capable of increasing airway PGI_2 levels, such as PARs, may prove to be useful in the treatment of asthma.

In addition to inhibition of Th_2 cells, it is likely that induction of T_{reg} would reduce airway inflammation in allergic asthma (McGee and Agrawal, 2006; Bohle *et al.*, 2007; Adcock *et al.*, 2008; Burchell *et al.*, 2008; Jin *et al.*, 2008). In this context, PAR_2 activation induced the maturation of immature murine DCs (iDCs) into mature DCs (mDCs) (Fields *et al.*, 2003). In addition, recent evidence suggests that PGE_2 may induce immune tolerance via induction of T_{reg} (Li *et al.*, 2008; Muthuswamy *et al.*, 2008). Pulmonary iDCs exposed to PGE_2 during maturation resulted in mDCs that attracted T_{reg} with increased affinity (Muthuswamy *et al.*, 2008). This effect was mediated by PGE_2-induced hypersecretion of CCL22, a proposed selective T_{reg} chemokine (Curiel *et al.*, 2004; Muthuswamy *et al.*, 2008). IFN-α ablated this effect, suggesting that

if viral infection were concomitant, an effective immune response would still develop (Muthuswamy *et al.*, 2008). Neither PGE_2 nor lipopolysaccharide (LPS) alone induced nearly as strong an effect; co-stimulation was necessary for hypersecretion of CCL22. This suggests a potential mechanism to explain the allergy resistance conferred by early life exposure to LPS (Cochran *et al.*, 2002).

Potential role of PARs in neutrophilic inflammation

Elevated numbers of neutrophils are a characteristic feature of numerous inflammatory lung diseases, including chronic obstructive pulmonary disease (COPD) and acute respiratory distress syndrome (ARDS), as well as certain forms of asthma (Pettersen and Adler, 2002; Jeffery, 2004; Kamath *et al.*, 2005; Cepkova and Matthay, 2006; Noma *et al.*, 2008). Signals for the influx of neutrophils into the lung are likely to include LTB_4 and IL-8, whose levels become elevated in response to inflammatory stimuli such as bacterial LPS. While the exact effects of LPS exposure in asthma remain to be elucidated, it appears to be involved in the development and severity of asthma (Michel, 2003). LPS exposure early in life may provide some protection against developing asthma; however, in established asthma, LPS exposure levels appear to be correlated with disease severity (Lapa e Silva *et al.*, 2000; Cochran *et al.*, 2002; Jung *et al.*, 2006; Kim *et al.*, 2007). Inhibiting neutrophil influx in asthmatics may be useful as neutrophils cause significant damage to the airways and are associated with severe and treatment-resistant forms of the disease (Delclaux *et al.*, 1997; Pettersen and Adler, 2002). Additionally, ARDS and COPD are also characterized by intense neutrophilia and may benefit from agents that reduce neutrophil influx into the lung. In this context, activators of PAR_2 reduced the airway neutrophilia associated with LPS exposure in mice (Moffatt *et al.*, 2002; Saleh *et al.*, 2008). PGE_2 also inhibited LPS-induced neutrophilia, indicating this product as a likely mediator of PAR_2-AP-induced inhibition of LPS-induced neutrophilia (Goncalves de Moraes *et al.*, 1996; Saleh *et al.*, 2008). Isolated bronchi from LPS-treated rats showed increased relaxation in response to PAR_2-APs, as well as increased PGE_2 release (Morello *et al.*, 2005). Thus PAR_2-mediated reductions in LPS-induced neutrophilia may be mediated by PGE_2; however, this remains to be shown experimentally. As effective medications for both ARDS and COPD are lacking, exploration of the possible benefits of novel therapeutic strategies such as activators of PARs is opportune.

Respiratory tract virus-induced exacerbations of asthma are typically associated with airway neutrophilia (Dougherty and Fahy, 2009). PAR activators can modulate host responses to respiratory tract viral infection, although the role of prostaglandins in these responses is unknown. For example, a recent study using cultured human monocytes revealed that PAR_2-APs were able to increase IFN-γ-induced effects, resulting in lower titres of influenza A virus, indicating a potentially protective role of PAR_2 activation during the progression of influenza A virus infection (Feld *et al.*, 2008). There are no published reports of the *in vivo* effects of PAR activation on the time-course of a respiratory tract viral infection. Nevertheless, influenza A virus infection in mice has been associated with elevated epithelial PAR expression, and augmented PAR_2-mediated inhibition of methacholine-induced bronchoconstriction (Lan *et al.*, 2004). In non-respiratory systems, PAR_1-APs have been shown to increase the viral infectivity of herpes simplex virus in human foreskin fibroblasts and human umbilical vein endothelial cells; however, neither PAR_2 nor PAR_4-APs increased viral infectivity (Sutherland *et al.*, 2007).

Changes in the levels or activity of the enzymes involved in the synthesis of prostaglandins can alter the host response to respiratory tract viral infection. Carey and coworkers (2005) revealed that deficiency of COX-1 is associated with an enhanced inflammatory response and earlier increases in the levels of the pro-inflammatory cytokines TNF-α and IL-1β (Carey *et al.*, 2005). In contrast, deficiency of COX-2 resulted in reduced inflammation and pro-inflammatory cytokine release, reduced morbidity and enhanced survival (Carey *et al.*, 2005). Thus, COX-1 and COX-2 appear to exert important but contrasting effects on the host immune response to influenza viral infection, which may be due to altered production of prostaglandins and leukotrienes. In a related study, mice that overexpress PGI_2 synthase selectively in bronchial epithelium had less respiratory syncytial virus-induced illness (Hashimoto *et al.*, 2004). In contrast, IP receptor-null mice showed increased mortality, weight loss and viral titers (Hashimoto *et al.*, 2004). This is consistent with earlier reports that exogenous PGI_2 administration greatly enhanced survival of mice exposed to viral infection and interestingly, this effect was reduced by COX inhibition (Zavagno *et al.*, 1987). As PARs increase prostaglandin production, more research is justified to examine the *in vivo* effects of PAR activators during viral infection of the lungs.

Potential role of PARs in pulmonary fibrosis

Pulmonary fibrosis is a characteristic pathologic feature of many lung diseases, including asthma, ARDS, COPD and idiopathic pulmonary fibrosis (Wynn, 2007). Fibrosis is difficult to reverse pharmacologically and is a major factor in the morbidity and mortality associated with these lung diseases (Rogliani *et al.*, 2008). Pulmonary fibrosis is a complex process involving varying extents of epithelial and endothelial injury, a state of hypercoagulation, fibroblast activation and differentiation, epithelial-mesenchymal transition, fibrocyte recruitment, extracellular matrix deposition, angiogenesis and aberrant repair mechanisms (Scotton and Chambers, 2007). A key cell-type involved in extracellular matrix deposition is the myofibroblast, whose numbers are elevated in fibrotic disease due to increased proliferation and decreased apoptosis. IL-4, IL-5 and IL-13 are important cytokines in pulmonary fibrosis as they induce release of active transforming growth factor-β (TGF-β), a potent fibrotic agent, capable of causing fibroblast proliferation and inflammatory cell recruitment through MCP-1 activation of CCR2 (Strutz, 2001; Szardening-Kirchner *et al.*, 2008). Along with these potent fibrotic effects, however, TGF-β is also involved in T_{reg} cell differentiation (Huber and Schramm, 2006; Wahl, 2007; Chen *et al.*, 2008). T_{reg} cells release IL-10, which suppresses inflammation and inhibits fibrosis, making their induction an attractive target for the treatment of fibrosis (Holsti *et al.*,

2004; Nakagome *et al.*, 2006; Couper *et al.*, 2008). The pathogenesis, aetiology and regulation of pulmonary fibrosis have recently been expertly reviewed (Wilson and Wynn, 2009). Here we will focus on the role of PARs and prostaglandins in pulmonary fibrosis.

Activation of the coagulation cascade, with the resultant generation of coagulation proteases such as thrombin, plays a central role in acute and chronic phases of fibrotic lung disease. For example, continuous infusion of a direct thrombin inhibitor significantly reduced lung collagen accumulation in a bleomycin model of pulmonary fibrosis (Howell *et al.*, 2002). A prominent role for PAR_1 in this model was subsequently established in studies showing that gene knockout of PAR_1 was protective against bleomycin-induced fibrosis (Howell *et al.*, 2005). It is not entirely clear how thrombin promotes fibrosis, although it induces PAR_1-dependent fibroblast to myofibroblast proliferation dependent upon PKC-α (Bogatkevich *et al.*, 2001), and inhibits apoptosis of fibroblasts through a PKC-ε-dependent mechanism (Bogatkevich *et al.*, 2005). Recent studies indicate that PAR_4 may also play a profibotic role. Stimulation of epithelial PAR_4 with thrombin or a PAR_4 AP-induced epithelial-mesenchymal transition, as evidenced by changes in cell morphology and changes in the expression of epithelial (e-cadherin) and myofibroblast (α-smooth muscle actin) markers (Ando *et al.*, 2007). In contrast, there is evidence that PAR_4 may suppress pulmonary fibrosis by countering PAR_1-stimulated proliferation of fibroblasts. In these studies, exposure of human primary bronchial fibroblasts to pro-inflammatory stimuli induced expression of functional PAR_4 on fibroblasts (Ramachandran *et al.*, 2007). In these TNF-α-stimulated fibroblasts, thrombin no longer induced proliferation, and a PAR_4-AP caused a reduction in fibroblast cell number (Ramachandran *et al.*, 2007). In this setting, a specific PAR_1-AP retained its mitogenic effects, indicating that thrombin activation of PAR_4 appears to suppress thrombin-mediated PAR_1 signalling (Ramachandran *et al.*, 2007). Furthermore, induction of PAR_4 expression enables cathespin G signalling, a proteinase that silences PAR_1 and PAR_2 but activates PAR_4. Interestingly, in TNF-α-treated fibroblasts, tryptase silenced PAR_2, rather than activating it (Ramachandran *et al.*, 2007).

Intratracheal administration of APC, a coagulation cascade inhibitory protein, is protective in bleomycin-induced pulmonary fibrosis (Yasui *et al.*, 2001). This is of particular interest because APC can activate PAR_1 via its coreceptor, the endothelial cell protein C receptor (Riewald *et al.*, 2002). Furthermore, this group has introduced the concept that when activating PAR_1, APC can stimulate signalling pathways distinct from those activated by thrombin, that is, the paradoxical condition that these two key coagulation proteases can mediate opposite effects on endothelial biology through the same receptor, PAR_1 (Riewald and Ruf 2005; Schuepbach *et al.*, 2008). Thus, stimulation of PAR_1 could inhibit or enhance fibrotic effects, depending on the method of activation.

While PAR_1 activation is typically associated with the development of fibrosis, the prostanoids PGE_2 and PGI_2 are potent anti-fibrotic agents. Suppression of COX activity increases bleomycin-induced fibrosis in mice (Keerthisingam *et al.*, 2001; Bonner *et al.*, 2002), and CCR2-null mice are protected from bleomycin-induced fibrosis, due to increased PGE_2 production from airway epithelial cells (Moore *et al.*, 2001; 2003; Lama *et al.*, 2002). EP_2 receptor activation by PGE_2 inhibits fibroblast proliferation and migration, transition to myofibroblast and collagen synthesis (Kolodsick *et al.*, 2003; Moore *et al.*, 2005; White *et al.*, 2005). PGI_2 also appears to be anti-fibrotic, with IP receptor-null mice being more susceptible to bleomycin-induced lung fibrosis (Lovgren *et al.*, 2006). Interestingly, in these studies, E-prostanoid receptor-null and mPGES-1-null mice did not exhibit any increase in bleomycin-induced fibrosis (Lovgren *et al.*, 2006). Consistent with this, no increase in bleomycin-induced fibrosis was observed in COX-2-null mice, despite lung dysfunction (Card *et al.*, 2007).

PAR activation induces PGE_2 release from fibroblasts, which down-regulate PAR_1 expression on these cells via a negative feedback loop (Sokolova *et al.*, 2005; 2008). PAR_2-mediated generation of PGE_2 by airway epithelial cells (Lan *et al.*, 2001) may also suppress fibroblast PAR_1 expression. Together, these findings indicate that PAR-mediated PGE_2 production within the airways may inhibit fibrosis through a number of mechanisms – indirectly via PGE_2-mediated suppression of fibroblast PAR_1 expression and directly by inhibiting fibroblast function as described above.

In summary, it appears that antagonists of PAR_1 may yield effective therapies in fibrosis. The role of other PAR subtypes is less clear with preliminary data suggesting that PAR_2-mediated generation of PGE_2 and PGI_2 inhibits fibrosis. Thus, a combination of PAR_1 antagonists, PAR_2-APs and protease inhibitors may be beneficial in suppressing fibrosis, although the specific pharmacological agents required to fully test this hypothesis are still in the development stage.

PAR interactions with respiratory system pharmacotherapies

Glucocorticoids are the mainstay therapy for inflammatory airway diseases and are likely to remain so for the foreseeable future. Thus, it is necessary for any new medications to maintain therapeutic activity when co-administered with glucocorticoids. As glucocorticoids suppress many of the pathways of PGE_2 production, the interaction between dexamethasone and PAR_2-APs has been investigated. While dexamethasone suppressed SLIGRL-induced increases in PGE_2 in cell culture, long- or short-term dexamethasone did not affect the PGE_2-dependent, SLIGRL-induced relaxation of isolated tracheal preparations (Saleh *et al.*, 2008). Furthermore, dexamethasone pre-treatment did not ablate PAR_2-AP-induced reductions in LPS-induced neutrophilia in mice (Saleh *et al.*, 2008). Interestingly, glucocorticoids were able to suppress PAR_2-mediated MMP-9 release from epithelial cells *in vitro*; however, this effect has not been examined *in vivo* (Vliagoftis *et al.*, 2000). This raises the possibility that glucocorticoids may be able to suppress PAR-mediated inflammatory pathways, but not anti-inflammatory pathways, although additional *in vivo* studies are required to confirm this. Although the interaction between PAR_2-APs and glucocorticoids has not been evaluated in other models of airway inflammation, these findings indicate that agents capable of increasing

endogenous PGE_2 production are likely to retain their effectiveness in the presence of glucocorticoids.

Other therapeutic agents also modulate PAR-mediated PGE_2 production in the airways. Inhibition of prostaglandin metabolism by the thiazolidinedione compound rosiglitazone (Cho and Tai, 2002) augmented PAR_2-mediated increases in PGE_2 levels and relaxation of isolated tracheal preparations (Henry *et al.*, 2005). Whether these effects persist *in vivo* is unknown, but warrants further investigation. Rosiglitazone is more widely recognized as an activator of PPAR-γ, and this class of drug alters a variety of inflammatory processes within the airways (Belvisi *et al.*, 2006; Ward and Tan, 2007). Further studies are required to investigate the potentially useful anti-inflammatory effects produced by combinations of glucocorticoids, PARs and PPAR-γ agonists (Belvisi *et al.*, 2006; Usami *et al.*, 2006).

Conclusions

While PARs are capable of inducing a wide range of inflammatory processes and cytokines within the lung, PAR-mediated prostaglandin production represents a distinct anti-inflammatory pathway. In the case of allergic inflammation, activation of PARs on epithelial cells causes PGE_2 production, resulting in lowered inflammatory cell recruitment and airway hyperresponsiveness, with PAR_2 being the most prevalent inducer of PGE_2 release (De Campo and Henry, 2005). Thus, inhaled agonists of PAR_2 may prove to be useful agents in the treatment of allergic airway inflammation. Additionally, the inhibition of neutrophil recruitment by PAR_2 is also likely to be prostaglandin-dependent. Coupled to their other anti-inflammatory properties, this effect would likely prove useful in the treatment of inflammatory airway diseases characterized by intense neutrophilia, such as ARDS, COPD and some forms of asthma (Moffatt *et al.*, 2002). Additionally, *in vivo* retention of anti-inflammatory effects upon concomitant glucocorticoid treatment makes PAR-mediated prostaglandin production an even more attractive target for the development of inflammatory airway disease treatments (Saleh *et al.*, 2008). Further investigation is therefore justified to determine whether PAR-mediated anti-inflammatory effects are retained in other models of airway inflammation *in vivo*. Additionally, the emerging role of PGI_2 in lung inflammation and its production by PARs warrant further investigation.

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